Laboratory journal igem team 2016

13.06

Eivind, Daniel and Monika

We started preparing supercompetent DH5α-cells, using the «making chemically supercompetent cells»-protocoll

14.06

Eivind

Inoculated DH5α-cells from the overnight culture into 100mL Psi-medium. Put to incubation at 37°C, 200 rpm.

OD measurements	
9:15	0,098
10:15	0,185
10:45	0,345
10:55	0,378
11:05	0,408

The cells were harvested at 11:05 and centrifuged at 4000 RPM, 4°C for 5 minutes. They were washed in TFb1, centrifuged and resuspended in TFB2.

Cells in eppendorfs frozen rack 815

15.06

Monika, Mats, Eivind, Nikolay

Prepared antibiotics:

Antibiotics	Concentration [mg/mL]
Kanamycin	50
Ampicillin	50
Chloramphenicol	35

Made agar-plates with antibiotics. Also prepared buffers.

Inoculated B0034(**RBS**), E1010 (**RFP**), B0015 (**terminator**), K608351 (**CI**), pFF706, pFF745 after transformaing one colony from the transformation plates to fresh plates into LB-medium with antibiotics. The cultures and plates were incubated at 37°C over night.

16.06

Mats, Eivind, Nikolay

Isolated plasmids from overnight cultures using Wizard Mini Prep set.

Nano drop	Conc [mg/mL]	260/280	260/230
pFF745	23,4	1,79	0,83
pFF706	29,5	1,71	0,78
RFP	44,6	1,75	1,09
T	33,8	1,79	1,15
CI	47,9	1,88	1,42
RBS	21,8	1,96	1,04

Cut CI, RFP with EcoRI and SpeI

Cut RBS, T with EcoRI and XBaI

20 µL reaction: 2 µL 10x CutSmart

	5 µL RBS	2 µL CI	3 µL T	2 µL RFP
H_2O	12,5	15,5	14,5	15,5
Enzyme	0,5	0,5	0,5	0,5

All restriction reactions were left overnight at 37°C.

Inoculated pCas and pTargetF into 10 mL LB medium with Spec/Kan selection. Cultures were incubated at 30°C (pCAs) and 37°C (pTargetF) overnight.

Inoculated 10 mL Psi-medium with DH5α cells from glycerol stock. Put to incubation at 37°C, 200 RPM.

17.06

Competent cell OD at t_0 = 0,1 L, t_{20} = 0,16, t_{40} = 0,24, t_{65} = 0,347, t_{70} = 0,388

Taken at OD 0,388

Standard protocoll performed

Performed gel electrophoresis on CI, RFP → OK and on RBS, T → FAIL → XbaI not functioning

20.06

Eiving, Nikolay

Another digestion with XbaI and EcoRI was attempted for RBS and terminator. ONly one cut was detected. This is thought to be a result of DH5α dam- activity, which methylates the adenine base in «GATC»-sequences. Since «GATC» is part of the XbaI-sequence in old biobricks, restriction digest is blocked in DH5α-grown plasmids.

21.06

Eivind

Transformed RBS and transcription terminator into ER2925 cells using heat shock transformation (1 µL DNA) The transformation mixes were plated out onto Cm-plates and left at 37°C over night.

A culture for making ER2925 cells with supercompetence was started from a 3 year old stock of supercompetent ER2925 cells. The culture was left at 37°C, 200 rpm overnight.

22.06

Eivind

Thousands of transformants had come up over night for both transformations.

ER2925 OD_{600}:

08:45 – 0,086

09:20 – 0,156

09:50 – 0,214

10:30 – 0,337

10:40 – 0,398

Prepared chemically supercompetent ER2925 cells. Put to storage in -80°C freezer in rack 8, box 6

23.06

Monika, Eivind, Mats

Restriction digest of DNA

10 µL RBS	10 µL Terminator
2 µL CutSmart Buffer	2 µL CutSmart Buffer
1 µL XbaI	1 µL XbaI
1 µL EcoRI-HF	1 µL EcoRI-HF
5 µL distilled water	5 µL distilled water

2 hours incubation 37 °C

Separated cut DNA on 2% agarose gel, but no DNA was visible in the RBS or terminator wells.

Set up new restriction reactions overnight

24.06

Eivind, Astri

Purified genes from gel (terminator, RBS, CI, RFP)

Ligated CI + RBS and RFP + terminator

Transformed both ligate mixes and positive pluss negative control into ER2925 cells.

27.06

Eivind, Astri

Made Cm-agar plates

Growth on all plates. Realized that ER2925 had the same antibiotice-resistance as we were using. Preparing new backbone. Transformed pSB2K3 into DH5α cells, plated onto kanamycin plates.

Digested RBS and terminator with EcoRI-HF and PstI-HF.

Digested CI and RFP with EcoRI-HF and SpeI.

28.06

Eivind, Astri

Digested RBS with SpeI and PstI

Digested RFP with XbaI and PstI

Did gel electrophoresis on digested RBS, terminator, Ci and RFP (from yesterday)

Inoculated pSB2K3 overnight

29.06

Eivind, Astri

B0034 (RBS): 12 bps

E1010 (RFP): 706 bps

B0015 (term): 129 bps

K608351 (CI): 948 bps

1 µL backbone DNA + 3 µL insert DNA

Cut out RBS (SpeI + PstI) and RFP (XbaI + PstI)

Purified RFP, CI, terminator and RBS from gel. Ligated RBS and RFP. Transformed RBS/RFP into DH5α (with negative).

30.06

Astri

RFP (X and P) empty. pSB2K3 (E and P) empty.

Terminator + pSB2K3 (0,5 µL + 0,5 µL → pSB2K3 empty)

LIgate RBS (S+P) and RFP (X+P)

Ligate RBS (E+P) and pSB2K3 (E+P)

Ligate term (E+P) and pSB2K3 (E + P)

Divided «10xBuffer for T4 DNA ligase with 100mM ATP) into 7 eppendorf tubes with 50 µL in each (+ in original tube).

Tried new gel recovery method (warm up elution buffer to 50^{O}C, centrifuge after wash without anything added) → gave good yield.

Tried new ligation method with incubation at 4^{o}C for 1-4 hours.

Transformed RBS+RFP, RBS+pSB2K3 and terminator + pSB2K3 in DH5α-cells

01.07

Astri

Resuspended DNA pSB4A5 from well (2J, plate 4, 2016) and transformed into DH5α cells.

Had colonies on RBS+ pSB2K3 and terminator + pSB2K3 plates → doing overnight culture

02.07

Astri

Miniprepped RBS + pSB2K3 and terminator + pSB2K3.

Moved plate with pSB4A5 colonies to fridge

04.07

Astri, Monika

Transformed RBS and terminator on kanamycin babckbone into ER2925.

Put pSB4A5 in overnight culture.

05.07

Astri

Given up all hope of growth on the RBS+RFP plates.

Pelleted and miniprepped the pSB4A5 overnight culture

	260/280	260/230	ng/µL
pSB4A5	2,5	0,01	5,2

Digested pSB4A5 with PstI-HF and EcoRi-HF in CutSmart buffer. Put to incubation overnight 37^{o}C.

DNA	30 µL
Buffer	6 µL
Restriction enzyme	1 µL (0,5 + 0,5)
water	13 µL

Put pSB4A5, RBS(pSB2K3 in ER2925) and Terminator (pSB2K3 in ER2925) in overnight culture 37oC, 205 rpm

06.07

Monika, Astri

Gel electrophoresis samples: pSB4A5 50 µL + 10 µL 6 x DNA dye -> 60 µL

0,5 µL Gene ruler 1kb Plus + 19,5 µL H2O + 4 µL 6 xDNA dye -> 24 µL

Electrophoresis 60 min 70 v gel green agarose 0,8%

Miniprepped pSB4A5, RBS (pSB2K3, ER2925) and terminator (pSB2K3, ER2925)

	260/280	260/230	ng/µL
RBS	1,97	1,37	23,0
Terminator	1,84	2,06	341,8
pSB4A5	1,86	2,07	310,3

pSB2K3 in freezer

Digestion of terminator, RBS and pSB4A5 with cutsmart buffer

	Terminator	RBS	pSB4A5
DNA	3 µL	30 µL	3 µL
Buffer	2 µL	6 µL	2 µL
Restriction enzyme	1 µL (0,5 XBaI + 0,5 EcoRI)	1 µL (0,5 XBaI + 0,5 EcoRI)	1 µL (0,5 PstI + 0,5 EcoRI)
water	14 µL	13 µL	14 µL

07.07

Monika, Astri

Gel electrophoresis on terminator, RBS and pSB4A5. Used zymoclean gel DNA recovery kit on the three samples

RBS: 0,280g → 840 µL buffer

pSB4A5: 0,331 g → 993 µL buffer

T: 0,253g → 765 µL buffer

Heated elution buffer to 70*C before added.

	260/280	260/230	ng/µL (dil 1:4)
RBS	0,65	-0,01	2,4
Terminator	1,08	-0,01	3,0
pSB4A5	1,53	-0,01	14,9

Digeston:

	Terminator	RBS
DNA	3 µL	10 µL
Buffer	2 µL	2 µL
Restriction enzyme	1 µL (0,5 XBaI + 0,5 EcoRI)	1 µL (0,5 XBaI + 0,5 EcoRI)
water	14 µL	7 µL

08.07

Monika

Prepared digested samples to electrophoresis

1 µL ladder 1 kb Plus + 19 µL h2O + 4 µL dye

20 µL RBS EcoRi, XBaI from 07.07 + 4 µL dye

20 µL T EcoRI, XBaI from 07.07 + 4 µL dye

20 µL RBS EcoRI, XBaI from 06.07 + 4 µL dye

11.08

Astri

Transformed 2 new RBS into DH5α-cells. B0032(2016, plate 2, 2J) and B0030 (2016, plate 4, 4G).

Plate don CM-plates and incubated overnight

12.07

Monika, Astri

HAd colonies on plates from yesterday. Put in overnight culture 37*C, 225 rpm. Put B0030-plates back to incubation to see if more colonies show up (had 2 today).

13.07

Monika, Astri

Miniprepped RBS: B0030 and B0032

RBS	260/280	260/230	ng/µL
B0030	1,69	1,17	177,8
B0032	1,78	1,54	228,6

Resuspended new terminator (BBa_B0014, pSB1AK3)(2011:P2:24C) and transformed into DH5α.

Restrictive digestion of RBS B0030 and B0032 with XBaI and EcoRI

	B0030	B0032
DNA	2 µL	3 µL
Buffer	2 µL	2 µL
Restriction enzyme	1 µL (0,5 XBaI + 0,5 EcoRI)	2 µL (0,5 XBaI + 0,5 EcoRI)
water	15 µL	14 µL

14.07

Monika, Astri

Electrophoresis of RBS B0030 and RBS B0032 (digested

RBS B0030 20 µL + 4µL 6xLD → 24 µL

RBS B0032 20 µL + 4 µL 6xLD → 24 µL

Gene ruler 1 kb Plus 1µL + 19 µL H2O + 4 µL 6xLD → 24 µL

70A, 2x40 min

Weight of cut DNA fragments

B0030: 0,070g → 210 µL buffer

B0032: 0,120g → 360 µL buffer

DNA purification with Zymoclean Gel DNA recovery kit. DNA concentration measured:

RBS	260/280	260/230	ng/µL (dilu 1:4)
B0030	1,47	0,76	10
B0032	2,06	0,71	9,2

Restriction digest of CI (27.06 53,4 ng/µL) with EcoRI-HF and SpeI

CI 2 µL, EcoRI-HF and SpeI-HF 0,5 µL each, cutsmart buffer 2 µL, H2O 15 µL

Incubated overnight

Made overnight culture of B0014 (terminator). Transformed ligated CI + RBS (both B0030 and B0032) into DH5α. PLated overnight

15.07

Astri

Miniprepped new terminator (B0014)

Colonies on both CI + RBS plates. Put in fridge for further work on Monday.

B0014: 153,4 ng/µL; 1,84 (260/280); 1,72 (260/230)

18.07

Monika

Electrophoresis of CI digested with EcoRI and SpeI

20 µL CI + 4 µL 6xDNA dye

19 µL H2O + 1 µL 100bp Plus DNA ladder + 4 µL 6x DNA dye

Purification

Overnight culture of RBS(B0030) and Ci in DH5α, RBS(B0032) and CI in DH5α in 10 mL LB medium and 10µL CM. Overnight 37*C, 225 RPM.

19.07

Monika, Astri

Miniprepped both ligations

Concentration after miniprepped:

	260/280	260/230	ng/µL
B0030 + CI	1,75	1,34	117,9
B0032 + CI	1,85	1,73	255

Digested RFP with EcoRI and SpeI. Digested terminator (new and old= with EcoRI and XBaI

	RFP	New T (B0014)	Old Terminator
DNA	1 µL	1,5 µL	3 µL
Buffer	2 µL	2 µL	2 µL
Restriction enzyme	0,5 µL EcoRI-HF + 0,5 µL SpeI-HF	0,5 µL EcoRI-HF + 0,5 µL XBaI	0,5 µL EcoRI-HF + 0,5 µL XBaI
water	16 µL	15,5 µL	14 µL

20.07

Monika, Astri

Gel electrophoresis on both terminators and RFP that was cut yesterday.

Used gel DNA recovery kit to purify DNA

	260/280	260/230	ng/µL
B0014	19,6	1,17	0,16
Old T	22,1	1,07	0,33
RFP	8,1	0,63	0,08 ☹

Ligation of old terminator and RFP, and new terminator and RFP

Transformed RFP + B0014(New T) and RFP + old T, and plate don agar plates. Incubated overnight at 37*C

21.07

Astri, Monika

Made overnight cultures for both ligations from yesterday

22.07

Astri

Miniprepped RFP + terminators (both ligations)

	260/280	260/230	ng/µL
RFP + old T	1,85	2,21	308,5
RFP + new T	1,81	1,92	220

Made new agar plates with Kanamycin.

25.07

Monika, Mats

Restrictive digestion of ligated plasmids

	CI + RBS (B0030)	CI + RBS (B0032)	RFP + new T	RFP + old T
DNA	1 µL	2,5 µL	2 µL	3 µL
Buffer	2 µL	2 µL	2 µL	2 µL
Restriction enzyme	0,5 µL EcoRI-HF + 0,5 µL SpeI-HF	0,5 µL EcoRI-HF + 0,5 µL SpeI-HF	0,5 µL EcoRI-HF + 0,5 µL XBaI	0,5 µL EcoRI-HF + 0,5 µL XBaI
water	16 µL	14,5 µL	15 µL	14 µL

26.07

Monika, Mats, Astri

Gel electrophoresis of digested plasmids in 0,8% GelGreen

CI + RBS (B0030) 20 µL + 4 µL 6x DNA loading dye

CI + RBS (B0032) 20 µL + 4 µL 6x DNA loading dye

New terminator + RFP 20 µL + 4 µL 6x DNA loading dye

Old terminator + RFP 20 µL + 4 µL 6x DNA loading dye

Ladder: GeneRuler 1 kb Plus 1 µL + 19 µL H2O + 4 µL 6x DNA loading dye

Picture of gel + cut out DNA from gel

Purified with gel DNA recovery kit

	260/280	260/230	ng/µL
CI + RBS(B0030)	1,76	0,03	16
CI + RBS(B0032)	2,12	0,11	29,2
RFP + new T	2,42	0,52	47,7
RFP + old T	1,76	0,09	41,7

27.07

Monika

Ligation:

1) CI + RBS B0030 with RFP + B0014(terminator)
   CI + RBS B0030 3 µL
   RFP + B0014 1µL
   10xT4DNA ligase buffer 2 µL
   T4 DNA ligase enzyme 1 µL
   Nuclease free water 13 µL
2) CI + RBS B0030 with RFP + old terminator
   CI + RBS B0030 3 µL
   RFP + old T 1µL
   10xT4DNA ligase buffer 2 µL
   T4 DNA ligase enzyme 1 µL
   Nuclease free water 13 µL
3) CI + RBS B0032 with RFP + B0014(terminator)
   CI + RBS B0032 3 µL
   RFP + B0014 1µL
   10xT4DNA ligase buffer 2 µL
   T4 DNA ligase enzyme 1 µL
   Nuclease free water 13 µL
4) CI + RBS B0032 with RFP + old terminator
   CI + RBS B0032 3 µL
   RFP + old T 1µL
   10xT4DNA ligase buffer 2 µL
   T4 DNA ligase enzyme 1 µL
   Nuclease free water 13 µL

28.07

Overnight cultures

1: KAN/DH5α E.coli/ CI+RBSB0030+RFP+(T)B0014
2: KAN/DH5α E.coli/ CI+RBSB0030+RFP+old T
3: KAN/DH5α E.coli/ CI+RBSB0032+RFP+(T)B0014
4: KAN/DH5α E.coli/ CI+RBSB0032+RFP+old T

29.07

Astri, Nikolay, Monika, Mats

- Culture 3 and 4 have a light pink color
- Miniprepped culture 1-4

	260/280	260/230	ng/µL
1	1,87	2,21	352,5
2	1,87	2,23	395,9
3	1,87	2,15	349,8
4	1,85	2,29	375,0

01.08
Astri, Mats

MAde new tests to see if the cells turns pink when exposed to 42^{O}C
Resuspended gBlocks gene fragments

Digeste the following with EcoRI and PstI
1: CI-RBS
2: P-RBS(INH)-RFP-T
3: RETRON BIOBRICK CI1
4: RETRON BIOBRICK CI2
5: RETRON BIOBRICK RBS1
6: RETRON BIOBRICK RBS2
7: RFP-T
8: pSB4A5
9: pSB2K3

DNA: 1µL
Buffer: 2µL
EcoRI: 0,5µL
PstI: 0,5 µL
Water: 16 µL
Tot: 20 µL

02.08
Mats, Nikolay, Astri

Gel electrophoresis on 1-9 from yesterday
Tried to check if the cells CI-RBS-RFP-term ligations we have turned red when expossing them to 43^{o}C.
The one ligation containing B0032 and B0014 have a red-ish glance to it. The others not so much.

Zymoclean gel DNA recovery

	260/280	260/230	ng/µL
RBS2 – 0,120g – 360µL	0,94	0,01	7,9
pSB4A5 – 0,190g – 570 µL	2,31	0,17	35,6
pSB2K3 – 0,120g -360µL	0,93	0,03	12,7
CI1 - 0,090g - 270µL	0,67	0,21	6,0
CI2 - 0,130g - 390µL	0,52	0,16	5,1
RBS1 - 0,140g - 420µL	0,52	0,08	5,2

Ligation of CI1, C2, RBS1, RBS2 with pSB2K3(as backbone)
pSB4A5 in freezer for tomorrow

Incubated cultures of B0032 + old T, B0030 + old T, B0032+B0014(T) and B0030+B0014(T) at 37^{o}C overnight.
The cultures was subjected to 43^{o}C for a couple of hours, to degrade CI protein.

Flourescence was measured (580 nm-615 nm)

	Fluorescence	OD
B0032, old T	43866*	1,324
B0030, old T	849	1,276
B0032, B0014(T)	36976*	1,341
B0030, B0014(T)	622	1,460

*Tomorrow we will do the same with samples not exposed to 43^{O}C as well

Transformation of all 4 ligations + negative into DH5α

03.08

Mats, Astri, Nikolay

Measured OD and flourescence in cultures at different temperatures

	Temp [^{o}C]	Fluorescence	OD	Well
B0032, old T	37	2120	0,836	B2
B0032, old T	42	2152	0,648	B4
B0030, old T	37	313	0,684	C2
B0030, old T	42	343	0,763	C4
B0032, B0014(T)	37	44054	0,815	B1
B0032, B0014(T)	42	42646	0,725	B3
B0030, B0014(T)	37	368	0,800	C1
B0030, B0014(T)	42	355	0,804	C3
LB-medium	37	270	-	D1
LB-medium	42	283	-	D2

Digestion of CI1, CI2, RBS1, RBS2, CI-RBS, RFP-T, and P-RBS-RFP-T with P and E.

No cultures on plates from yesterday. Left at 37oC to see if anything happens.

New floureecense test with heating directly in wells:

	Well	OD
B0032, old T	E1	0,805
B0030, old T	E2	0,726
B0032, B0014(T)	E3	0,667
B0030, B0014(T)	E4	0,734
LB-medium	D3	-

04.08

Prepared digestions from yesterday

Flourescence test:

	Well	OD
B0032, old T	F1	0,611
B0030, old T	F2	0,577
B0032, B0014(T)	F3	0,604
B0030, B0014(T)	F4	0,417
LB-medium	F5	-

Digested K-backbone (for 2 hrs). Totally forgot yesterday

Separation on gel.

	g	µL	ng/µL	260/280	260/230
CI1	0,265	795	37,5	3,80	0,55
CI2	0.091	273	6,8	0,71	0,04
RBS1	0,230	690	9,5	0,66	0,25
RBS2	0,239	717	4,2	0,5	0,09
CI-RBS	0,265	795	6,5	0,63	0,18
RFP-T	0,180	540	6,3	0,61	0,09
CI-RBS-RFP-T	0,233	699	23,1	2,48	0,08
pSB2K3	0,296	900	-	-	-

05.08

Astri, Mats

	Temp [°C]	OD	Well
30,14	37	0,610	G1
30, T	37	0,512	G2
32, 14	37	0,456	G3
32, T	37	0,557	G4
30,14	42, 1h	0,571	H1
30, T	42, 1h	0,681	H2
32, 14	42, 1h	0,627	H3
32, T	42, 1h	0,706	H4
30,14	42, 2h	0,898	A5
30, T	42, 2h	0,598	A6
32, 14	42, 2h	0,753	A7
32, T	42, 2h	0,558	A8

Ligation of CI1, CI2, RBS1 and RBS2 (each alone) with pSB2K3

LIgation of CI-RBS, RFP-T, and CI-RBS-RFP-T (each alone) with pSB4A5

Transformation of all ligations into DH5α

	Temp [°C]	OD
30,14	42, 3h	0,752
30, T	42, 3h	0,833
32, 14	42, 3h	0,761
32, T	42, 3h	0,714
30,14	42, 4h	0,691
30, T	42, 4h	gir
32, 14	42, 4h	0,685
32, T	42, 4h	0,505

-30,14 has a very different color after 4 hrs at 42°C!!:)

06.08

Astri

Had colonies on all plates. Made overnight cultures. Out three plates back for longer incubation due to few colonies so we may have backups.

07.08

Astri

Sentrifuged down the overnight cultures. Put pellets in freezer. Made new overnight culture of CI-RBS due to no culture from yesterday. Made overnight culture of CI-RBS-RFP-T for testing.

08.08

Mats, Astri

New flour.test with synthetic construct

	Temp [°C]	OD
0	42	0,924
1	42	1,103
2	42	1,017
3	42	1,173
4	42	1,130

Transformed TetR(BBa_R0040) and LacI(BBa_R0010) into DH5α-cells. Cm-resistance.

Miniprepp:

	ng/µL	260/280	260/230
CI1 + pSB2K3	428,1	1,85	2,25
CI2 + pSB2K3	487,8	1,83	2,24
RBS1 + pSB2K3	448,8	1,80	1,87
RBS2 + pSB2K3	517,2	1,83	2,30
RFP-T + pSB4A5	379,7	1,77	1,59
CI-RBS-RFP-T + pSB4A5	408,1	1,83	1,90

Set overnight culture of DH5α cells in PSI-medium

Prepared overnight cultures for flourescence testing 25 mL + 25 µL KAN/AMP stock solution

09.08

Astri

Made more DH5α-cells:

11:30 – 0,107

12:00 – 0,174

12:40 – 0,286

13:10 – 0,459

Overnight cultures of TetR, CI-RBS in pSB4A5 and LacI

10.08

Mats, Nikolay

Nanodrop:

LacI – 204,3 ng/µL

TetR – 276,1 ng/µL

Restriction digest

	ng/µL	VDNA [µL]	Venz[µL]	Cutsmart [µL]	H2O [µL]
LacI	204,3	5,1	S: 0,5 P: 0,5	1	12,9
tetR	276,1	3,7	S: 0,5 P: 0,5	1	14,3
RBS1	448,8	2,3	X: 0,5 P: 0,5	1	15,7
RBS2	517,2	2	X: 0,5 P: 0,5	1	16
CI1	428,1	2,4	X: 0,5 P: 0,5	1	15,6
CI2	487,8	2,1	X: 0,5 P: 0,5	1	15,9

Gel electrophoresis – FAIL!

Started overnight digestion

11.08

Mats

Gel electrophoresis

Zymoclean DNA recovery and nano drop:

TetR: 17,6 ng/µL

LacI: 24,3 ng/µL

RBSII: 18,7 ng/µL

NEW PLAN!!

Nanodrop of terminator on pSB2K3 Backbone

291,9 ng/µL

Digestion:

Method 1:

	ng/µL	VDNA [µL]	Venz[µL]	Cutsmart [µL]	H2O [µL]
CI1	8	15	E: 0,5 P: 0,5	1	3
CI2	5,1	15	E: 0,5 P: 0,5	1	3
RBS1	5,4	15	E: 0,5 P: 0,5	1	3
RBS2	5,7	15	E: 0,5 P: 0,5	1	3
pSB2K3	290,9	3,4	E: 0,5 P: 0,5	1	14,6

Method2:

	ng/µL	VDNA [µL]	Venz[µL]	Cutsmart [µL]	H2O [µL]
CI1	8,0	15	X: 0,5 P:0,5	1	3
CI2	5,1	15	X: 0,5 P:0,5	1	3
RBS1	5,4	15	X: 0,5 P:0,5	1	3
RBS2	5,7	15	X: 0,5 P:0,5	1	3
LacI	204,3	4,9	S: 0,5 P:0,5	1	13,1
tetR	276,1	3,6	S: 0,5 P:0,5	1	14,4

37^{o}C overnight.

12.08

Monika

Electrophoresis of cut pSB2K3, LacI and tetR in 0,8% agarose gel. 2x30 min – didn't work. Possibly because gel taken from the bottom of flask had much higher concentration. PCR cleaning of elements digested with E and P: CI1, CI2, RBS1, RBS2 and with X and P: CI1, CI2, RBS1, RBS2

Digestion of pSB2K3 with c = 338 ng/µL

13.08

Monika

Electrophoresis of cut sample pSB2K3 – FAILED!

Another attempt on digestion done.

15.08

Astri

Electrophoresis of cut sample pSB2K3

LIgation of CI1, CI2, RBS1 and RBS2 into pSB2K3

Ligation of CI1 and RBS1 into LacI and CI2 and RBS2 into tetR.

Transformation

Made two more batches of agar plates with K and A resistance, respectively.

16.08

Astri

Made overnight cultures of all transformations from yesterday

17.08

Miniprep of 7 overnight cultures (RBS1 + LacI had to be repeated

	ng/µL
CI1 + LacI	170,5
CI1 + pSB2K3	305,6
CI2 + tetR	286,1
CI2 + pSB2K3	205,7
RBS2 + tetR	191,9
RBS2 + pSB2K3	185,7
RBS1 + pSB2K3	100,8

18.08

Restriction digest of:

	VDNA [µL]	Venz[µL]	Cutsmart [µL]	H2O [µL]
CI1 + LacI	6	P: 0,5 S: 0,5	2	11
CI2 + tetR	3,5	X: 0,5 P: 0,5	2	13,5
RBS1 + LacI	5	P: 0,5 S: 0,5	2	12
RBS2 + tetR	5,5	X: 0,5 P:0,5	2	11,5

Miniprep of RBS1 + LacI: 340 ng/µL

19.08

Made new CM-agar plates

Electrophoresis failed

22.08

Repeated restriction digestion of CI1+LacI, CI2+tetR, RBS1+LacI, RBS2+tetR

	VDNA [µL]	Venz[µL]	Cutsmart [µL]	H2O [µL]
CI1 + LacI	6	P: 0,5 S: 0,5	2	11
CI2 + tetR	3,5	X: 0,5 P: 0,5	2	13,5
RBS1 + LacI	5	P: 0,5 S: 0,5	2	12
RBS2 + tetR	5,5	X: 0,5 P:0,5	2	11,5

Post-electrophoresis purification of:

	ng/µL
CI1 + LacI	28,1
CI2 + tetR	37,9
RBS1 + LacI	67,9
RBS2 + tetR	10,7

After purifying, material ready for ligation is storred in yellow box, place D1-4

23.08

Astri

Ligation of CI2+LacI and CI2 + tetR

Ligation of RBS1+LacI and RBS2 + tetR

Set to incubation at 4°C 11:40-16:30

Transformed ligation mixe into DH5α. LIgation mixes is stored in yellow box A7 and A8.

Also counted agar plates and pipette boxes. Numbers on yellow post-its. Please modify these when you use soemthing.

Incubated transformations and negative at 37°C.

24.08

Astri

Made overnight cultures of LacI-RBS1-tetR-RBS2 and LacI-CI1-tetR-CI2.

Put to incubation 37°C-225rpm

25.08

Eivind

Miniprepped the overnight cultures from 24.08. Put in freezer «RBS retron plasmid», «CI retron plasmid»

PCR verification:

1 LacI1-RBS2 ~2kB

2 LacI1-RBS1 ~1kB

3 TetR-RBS2 ~1kB

4 LacI1-CI2 ~2kB

5 LacI1-CI1 ~1kB

6 tetR-CI2 ~1kB

Pan PCR verification

26.08

Eivind

Gel separation of PCR products

05.09

Eivind

Gel verification of retron plasmid building blocks

08.09

Eivind

Sequencing of CI and RBS retron plasmids: CI, RBS

12.09

Eivind

After failed verification of retron biobricks and their building blocks, new ligations were performed:

CI1, CI2, RBS1, RBS2 all into (by them selves) pSB2K3

Additionally, tetR and LacI were digested overnight: tetR: E+P, tetR: S+P, LacI: E+P, LacI: S+P

13.09

Eivind

Gel separation of LaciI and TetR fragments

All fragments were of expected length and were excised and purified from gel slices.

Inoculated 4x10mL LB+Kan(50 µL/mL) with the ligation transformants for overnight cultures. Plates were put to storage in fridge.

Verification of synthetic retron parts: looks to be correct size for all fragments.

14.09

Eivind

Mini prepped overnight cultures and digested ~1µg for 2hours with X and P.

CI1, CI2 and RBS1 had correct fragment sizes.

RBS2 most likely failed ligation, resulting in the cutting pattern of pSB2K3

06.10

Monika

PCR: LacI, CI1, RBS1, tetR, CI2, RBS2

08.10

Astri

Transformed CI-RBS-RFP-T on pSB4A5 into DH5α-cells. Hopefully.

09.10

Astri

Got two kinds of colonies, red and white. Pu tone of eachto inoculation in PSI-medium. (15:30)

10.10

Astri

Started culture for supercompetent cells at 08.35.

Time	OD Red	OD White
10:10	0,09	0,087
11:00	0,158	0,168
11:30	0,254	0,259
11:43	0,283	0,284
12:00	0,365	0,374

I've marked the eppendorf (20 of them) with the white cultures: DH5α P 10/10

I've marked the eppendorf (5 of them) with the red cultures: DH5α PR 10/10

11.10

Monika

Cleaning of PCR products. Concentration after:

	Ng/µL
CI1	52,5
RBS1	75,6
TetR	53,9
LacI	52,6
CI2	73,4
RBS2	27,6

12.10

Astri, Eivind

Gel electrophoresis on PCR-parts. FAILED!

Re-did the PCR

Made CM and AMP resistant agar plates.

13.10

Eivind, Astri

Gel electrophoresis on PCR-parts. Looks great!

	Ng/µL
CI1	168,4
CI2	222,8
RBS1	171,7
RBS2	9,2
LacI	166,7
tetR	28,1

Made our own Gibson Assembly mix.

14.10

Astri

Gibson assembly:

Vgibs = 30µL

Vci1 = 4 µL

Vci2 = 4 µL

VtetR = 0,52µL

VlacI = 1,20 µL

Vh2o= 0,28 µL

Tot: 40 µL

Transformation of 5µL of gibson assembled plasmid into:

Normal DH5α + negativ: 1&2 Cm-res

DH5α P + negative: 3&4 A+Cm-res

DH5 PR + negative: 5&6 A+CM res

15.10

Astri

No colonies on DH5α or DH5αPR. Two small ones on DH5α. Making overnoght cultures with those.

Since there are two tiny colonies on the DH5α-plate I'm assuming the Gibson assembly worked, but the transformation didn't. Therefore, I'll do a new tranformation with the Gibson assembly from yesterday.

16.10

Eivind

Testing for Oslo team - Abs@486nm

Colonies on DH5α PR. Made overnight cultures. Digested

17.10

Astri, Eivind

Separation on gel.

Transformation